

CLAIMS

1. A method of producing a clustered array of one or more clonal copies of a single target molecule immobilised on a solid support, each cluster in said array being capable of resolution by optical microscopy, said method comprising providing clonal copies of a single target molecule within a vesicle or on the surface of a solid support within a chamber defined by a vesicle in contact with said solid support to produce clonal copies thereof and immobilising said copies on said solid support.
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- 10 2. A method according to claim 1 wherein said target molecule is a nucleic acid molecule.
- 15 3. A method according to claim 1 or claim 2 which comprises
 - a) providing a vesicle in contact with the surface of a solid support so as to define a chamber between said support and said vesicle, which chamber comprises a single target molecule including a functionality to effect immobilisation to the surface of said solid support,
 - 20 b) copying said target molecule on the surface of said support to produce copies of said target molecule forming said cluster.
- 25 4. A method according to claim 3 wherein said method comprises the step of first contacting with said support a single target molecule including said functionality to effect immobilisation to the surface of the support and subsequently contacting said vesicle with said support so as to provide a chamber between said support and said vesicle, which chamber comprises said single target molecule.
- 30 5. A method according to claim 3, wherein said method first comprises the step of contacting with said solid support a vesicle defining an enclosed chamber therein which chamber comprises therein said single target molecule.

6. A method according to any one of claims 3 to 5 wherein said functionality to effect immobilisation to the surface of said solid support is capable of interacting with a complementary capture moiety attached to the surface of said solid support.

5 7. A method according to claim 6 wherein said single target molecule is a nucleic acid molecule and said functionality to effect immobilisation to the surface of said solid support is a sequence of nucleotides in said nucleic acid molecule capable of hybridising to a nucleotide sequence in a complementary capture moiety which is a polynucleotide molecule attached to the surface of the solid support.

10 8. A method according to claim 7 wherein the capture moiety is an amplification primer.

9. A method according to claim 8 wherein step b) comprises amplification of the
15 single target nucleic acid molecule using said amplification primer.

10. A method according to claim 9 wherein said amplification is carried out using said amplification primer attached to the surface of the solid support and a second amplification primer in free solution within the chamber defined between said support
20 and said vesicle.

11. A method according to claim 10 wherein said target nucleic acid molecule (in single stranded form) includes a first known adaptor sequence at its 3' end and a second known adaptor sequence at its 5' end, wherein the first known adaptor sequence is capable
25 of hybridising to the amplification primer attached to the surface of the solid support and the complement of the second known adaptor sequence is capable of hybridising to the amplification primer present in free solution.

12. A method according to claim 1 or claim 2 comprising,
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a) providing at least one vesicle defining an enclosed chamber therein, said chamber including said single target molecule and one or more copies thereof,

5 b) contacting said vesicle with the surface of a solid support to effect localised immobilisation of said copies of the target molecule to the surface of said support, each of said copies including a functionality to effect said immobilisation to the surface of said solid support.

10 13. A method according to claim 12 wherein said target molecule is a nucleic acid molecule and said copies of said target molecule are copies produced by nucleic acid amplification inside said vesicle.

15 14. A method according to claim 12 or claim 13 wherein said copies of said target molecule are capable of being attached to said solid support via a functionality thereon capable of interacting with a complementary capture moiety having means to effect attachment to the surface of said support.

20 15. A method according to claim 14, wherein said target molecule is a nucleic acid molecule and said capture moiety comprises a nucleic acid molecule comprising a nucleotide sequence that is complementary to a nucleotide sequence on the copies of the target nucleic acid molecule.

25 16. A method according to claim 15, wherein said target nucleic acid molecule comprises an adaptor molecule of known nucleic acid sequence at one of its 5' and 3' ends.

30 17. A method according to any claim 15 wherein said target nucleic acid molecule includes an adaptor molecule of known nucleic acid sequence at each of its 5' and 3' ends.

18. A method according to claim 17 wherein said amplification step utilises a single primer species specific for said 3' adaptor molecule to produce a nucleic acid molecule having a sequence complementary to the target nucleic acid molecule.

19. A method according to any of claims 15 to 18 wherein said capture moiety comprises a hairpin oligonucleotide.
20. A method according to claim 19, wherein said hairpin oligonucleotide comprises a sequence its 3' end corresponding to that of the 5' adaptor molecule of the target nucleic acid molecule and a 3' blocking group.
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21. A method according to claim 20, wherein said hairpin oligonucleotide is present in the chamber of said vesicle, capture of the complementary copies of said single target molecule occurring prior to the fusion of said vesicle to said solid support.
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22. A method according to claim 20, wherein said hairpin oligonucleotide is present on the surface of said support, capture of the complementary copies of said single target molecule occurring subsequent to the contacting of said vesicle with said solid support.
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23. A method according to claim 21 or 22 wherein said vesicle further comprises a ligase enzyme to ligate the complementary copies of said target molecule to the 5' end of said hairpin incorporating a phosphate moiety.
- 20 24. A method according to claim 1 or claim 2 comprising,
 - a) providing at least one vesicle defining an enclosed chamber therein and one or more copies of said single target molecule within said vesicle, said vesicle comprising a linking molecule for linking said copies together in said vesicle, and
 - b) applying said linked copies of said target molecule to a solid surface to form an array of clustered arrays of said copies, said linking molecule comprising a functionality to effect attachment to the surface of said support.
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25. A method according to claim 24 wherein said linking molecule comprises a dendrimeric molecule, said one or more copies of said single target molecule being generated by providing on said dendrimeric molecule one or more copies of a single primer species complementary to a nucleotide sequence in the target molecule in the presence of a polymerase and appropriate dNTP molecules.
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26. A method according to claim 24 wherein said linking molecule comprises a polymerisation initiator attached to said copies.

5 27. A method according to any preceding claim, wherein said vesicle comprises an isolated chamber in a bulk phase, whose interface with the bulk phase prevents exchange of the single target molecule and copies thereof in the aqueous chamber with the bulk phase.

10 28. A method according to any preceding claim wherein said vesicle is formed from droplets of water emulsified in oil.

29. A method according to claim 27 wherein said vesicle is a liposome.

15 30. A method according to claim 27 wherein said vesicle is formed from a polyelectrolyte nanoshell.

31. A method according to claim 27 wherein said vesicle comprises an aqueous core and/or a shell formed by coacervation.

20 32. A vesicle for use in producing a clustered array of one or more clonal copies of a single target molecule, said vesicle comprising an enclosed chamber therein comprising either said single target molecule and/or copies thereof or the components to effect copying of said single target molecule or both, and which vesicle is formed from an isolated chamber in a bulk phase, whose interface with the bulk phase prevents exchange of the single target molecule and/or copies thereof with the bulk phase.

25 33. A vesicle according to claim 32, which vesicle is formed from droplets of water emulsified in oil.

30 34. A vesicle according to claim 32 which vesicle is a liposome.

35. A vesicle according to claim 32 which is formed from a polyelectrolyte nanoshell.

36. A vesicle according to any of claims 32 to 35 which has an aqueous core and/or a shell formed from coacervation.

5 37. A vesicle according to any of claims 32 to 36, wherein said single target molecule or copies thereof comprise a functionality capable of interacting with a complementary capture moiety having means to effect immobilisation of said target molecule or copies thereof to the surface of said solid support.

10 38. A kit for producing a clustered array of one or more clonal copies of a single target molecule which kit comprises a plurality of vesicles according to any of claims 32 to 37 and a solid support for contacting with said vesicles.

15 39. A kit according to claim 38, wherein said target molecule is a nucleic acid molecule.

40. A kit according to claim 38 or 39, wherein said capture moiety comprises a nucleic acid molecule comprising a sequence that is complementary to a sequence on the 20 target nucleic acid molecule or copies thereof.

41. A kit according to any of claims 38 to 40, wherein said target nucleic acid molecule comprises an adaptor molecule of known nucleic acid sequence at one of its 5' and 3' ends.

25 42. A kit according to any of claims 38 to 40 wherein said target nucleic acid molecule includes an adaptor molecule of known nucleic acid sequence at each of its 5' and 3' ends.

30 43. A kit according to claim 42 further comprising a first amplification primer capable of hybridising to said 3' adaptor molecule and a second amplification primer which is capable of hybridising to the complement of said 5' adaptor sequence, wherein the first

amplification primer includes a functionality which permits attachment to said solid support.

44. A kit according to claim 42 further comprising a single primer species specific for
5 said 3' adaptor molecule for use in an amplification step to produce a nucleic acid
molecule having a sequence complementary to the target nucleic acid molecule.

45. A kit according to any of claims 38 to 42 or 44 wherein said capture moiety
comprises a hairpin oligonucleotide.

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46. A kit according to claim 45, wherein said hairpin oligonucleotide comprises a
sequence its 3' end corresponding to that of the 5' adaptor molecule of the target nucleic
acid molecule and a 3' blocking group.

15 47. A kit according to claim 45 or 46, wherein said hairpin oligonucleotide is present
in the chamber of said vesicle.

48. A kit according to claim 45 or 46 wherein said hairpin oligonucleotide is present
on the surface of said solid support.

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49. A kit according to any of claims 46 to 48 further comprising a ligase enzyme to
ligate the 3' end of the complementary copy of said target molecule to the 5' end of said
hairpin incorporating a phosphate moiety.